Seizure (2007) **16**, 459—464

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SHORT COMMUNICATION

Pyridoxine induces non-specific EEG alterations in infants with therapy resistant seizures

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Received 1 October 2006; received in revised form 21 December 2006; accepted 20 February 2007

KEYWORDS
Pyridoxine dependent seizures;
Therapy resistant seizures;
Pyridoxine responsiveness;
Infant;
Amplitude;
EEG

Summary

Purpose: In infants with frequent therapy resistant seizures (TRS-infants), clinical detection of pyridoxine-dependency (PD) or -responsiveness (PR) occurs by empirical intravenous (IV) pyridoxine administration during recording of the EEG. However, in undiagnosed TRS-infants it is still unclear to what extent EEG alterations by pyridoxine-IV are attributable to PD/PR or to non-specific responses. Before EEG alterations by pyridoxine-IV can be ascribed to PD/PR, these non-specific responses should be excluded first.
Methods: In 10 TRS-infants under 1 year of age, we determined the EEG effect by pyridoxine-IV on the EEG-recording.
Results: After pyridoxine-IV administration, our data indicate declined (10—15%; $p < 0.05$) EEG-amplitudes and total power (magnitude/frequency-band) at frontal, central and centro-temporal electrodes.
Conclusion: In TRS-infants, pyridoxine-IV affects EEG-amplitude and -total power in a non-specific way, which does not identify PD/PR.

Introduction

Pyridoxine is a common cofactor involved in many metabolic pathways of the brain.[1] In a small, heterogeneous group of infants with therapy resistant

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1059-1311/$ — see front matter
doi:10.1016/j.seizure.2007.02.008

seizures (TRS-infants), pyridoxine administration may stop or attenuate seizures.[2] According to well-defined clinical criteria, this response can be characterized as pyridoxine-dependency (PD) or as pyridoxine-responsiveness (PR), respectively.[2] PD is characterized by: (1) seizures persisting despite administration of at least two anti-epileptic drugs, (2) seizure cessation after pyridoxine administration, (3) complete seizure control by pyridoxine monotherapy, and (4) seizure recurrence after pyridoxine withdrawal.[3] The term PR is reserved for infants that show reduced seizure activity by pyridoxine, but in whom the seizures do not meet all PD criteria.[2,4]

Different enzyme deficiencies may explain the effect by pyridoxine (or its active metabolite pyridoxal phosphate) on seizure activity,[5,6] including one in which shortage of pyridoxal phosphate is linked to an impaired conversion of (the excitatory amino acid) glutamate into (inhibitory) GABA. In a neonatal subgroup, this has been related to a mutation in the *PNPO* gene, encoding for pyridox(am)ine-5′-phosphate oxidase (which converts pyridoxine into pyridoxal phosphate).[7] As a consequence, empirical administration of pyridoxal phosphate (instead of pyridoxine) is increasingly recommended, especially in neonates and in children with West syndrome.[7–9] Another important PD-subgroup is ascribed to α-aminoadipine semialdehyde [ALDH7A1 (antiquitin)] deficiency. These patients can be diagnosed by mutation analysis of the ALDH7A1 gene[10] and by biochemical analysis [of pipecolic acid[11,12] and of alpha amino adipic semialdehyde (alpha-AASA)[10] concentrations (in blood/CSF and in urine, respectively)]. In these patients, PD is caused by chemical inactivation of pyridoxine phosphate (due to reaction with α-aminoadipine semialdehyde).[6,10] A similar chemical inactivation of pyridoxine is also involved in PD patients due to hyperprolinemia type II.[13]

Although these PD/PR-subgroups can be identified, time is needed for detection. Furthermore, incomplete diagnostic knowledge of other PD/PR-subgroups may still hamper fast and accurate recognition.[5,14] To facilitate recognition of PD/PR among TRS-infants, pyridoxine is empirically supplied according to different test-protocols. In TRS-infants with low seizure frequencies, PD/PR is clinically characterized by the response after oral pyridoxine administration (for at least 14 days) and subsequent withdrawal.[2] To optimize identification of PD/PR, EEG recordings before- and after-pyridoxine administration are preferably taken into account.[5] In TRS-infants with high seizure frequencies (or status epilepticus), a test dose of pyridoxine-IV is administered.[3] Within minutes after pyridoxine infusion, seizure activity may stop in PD/PR.[3,15] To allow fast and accurate detection of the response, pyridoxine-IV guidelines recommend simultaneous recording of the EEG.[2,3] In PD/PR patients, EEG recordings preceding pyridoxine administration may reveal focal (frontal, parietal or temporal) bursts of sharp slow wave activity (1–4 Hz) and bilateral synchronous discharges.[3] In PD/PR patients, this epileptic activity may disappear within 5 min after pyridoxine-IV administration[2] and the EEG amplitude may reduce or flatten.[1] Although rapid normalization of the EEG is generally expected,[5] normalization of the EEG may be incomplete[16] or delayed (for hours-days).[2,15] Especially in PD/PR children with associated morbidity such as asphyxia, intracranial hemorrhages or multi-system involvement[2] this could be the case.

In perspective of this clinical variability, EEG control data during pyridoxine-IV in TRS-infants (without PD/PR) seem important. However, such EEG control data have not been reported yet. For instance, it is still unknown whether pyridoxine-IV also induces EEG-alterations that do not identify PD/PR. Such "non-specific" effects should be taken into account before EEG-alterations are attributed to PD/PR. To evaluate whether such "non-specific" EEG-alterations occur, we studied EEG recordings in TRS-infants during an empirical test dose of pyridoxine-IV.

Methods

Patients

At the University Medical Center Groningen, we retrospectively collected digital EEG data of 10 TRS-infants (of less than 1 year of age) that received a clinical trial of pyridoxine-IV between 2001 and 2004. The medical ethical committee decided that, according to Dutch legislation, no approval is needed for this kind of retrospective data collection. Informed consent from the parents of all included infants was obtained. TRS was defined as intractable seizures despite administration of two first-line anti-epileptic drugs in adequate dosages.

Study design

Before and after administration of 100 mg pyridoxine-IV, EEG segments were digitally compared for average background amplitude. Thereafter, alterations of delta (0–3 Hz), theta (4–8 Hz) and beta (13–30 Hz) frequency-bands were separately assessed regarding amplitude, total-and relative-power. Total power reflects the magnitude of background activity for an individual frequency band. Relative power indicates the relative contribution of a frequency-band to the entire spectrum.[17] Both

before and 5—15 min after administration of pyridoxine-IV, two separate 1 min EEG segments with identical vigilance states were assessed. These states (asleep or quietly awake) were identified by EEG and clinical observation. Only EEG segments of identical vigilance states were compared.

Digital EEG recording and analysis

Digital EEG recording and analysis was performed using Brainlab Version 4.00-0.00 and Brain Vision Analyzer Version 1.030002. All digital EEGs involved a 21-channel recording according to the international 10/20 system. Bipolar recordings were evaluated at frontal (F3-F4), centro-temporal (T3-C3; C4-T4), central (C3-C4), and occipital (O1-O2) electrodes.

Selected segments before and after pyridoxine-IV were digitally compared regarding amplitude and total power of delta (0—3 Hz), theta (4—8 Hz) and beta (13—30 Hz) frequency-bands. For power calculations, a Fast Fourier Transform was applied.

To assess a potential shift in frequency-bands by pyridoxine-IV, relative power per frequency-band was calculated according to the formula:

$$p_{\text{relative}}[f_1, f_2] = \frac{p[f_1, f_2]}{p[0, 50]} \times 100\%$$

$p[f_1,f_2]$ is the total power in the frequency-band between f_1 and f_2 Hz.

For further reading on Power Analysis, see also ref. 17.

Statistical analysis

Regarding amplitude and power analysis, EEG segments before and after pyridoxine-IV were compared by the Wilcoxon signed rank test. Mann—Whitney *U* test was applied for EEG comparison between infants that were awake or asleep.

Results

Clinical data

Table 1 shows patient characteristics of the ten included infants. CSF GABA concentrations before pyridoxine administration were within the normal range (>2 ng/ml). Three neonates were born after severe or moderate fetal distress (cases 1, 3 and 7). Median age of seizure onset was at postnatal day 1 (range 0—10 months). Median duration between seizure onset and pyridoxine-IV was 14 days (range 1 day to 8 months).

Two patients (case 1 and 3) were clinically characterized as PR. Although seizures did not disappear by pyridoxine monotherapy alone,[2] both infants showed attenuated seizure activity by subsequent oral pyridoxine administration. In each infant, pyridoxine withdrawal was performed twice, resulting in seizure recurrence within 24 h (for detailed description of case 1, see also ref. 16). At 2 years of age, eight patients were still on anti-epileptic drugs and seizure control was achieved in five patients.

EEG findings

Pyridoxine-IV decreased amplitude ($p < 0.05$) at central, centro-temporal and frontal electrodes [C3-C4: median −13% (−49 to +15%; Fig. 1); C3-T3: median −15% (−58 to +5%) and F3-F4: median −11% (−64 to +19%)]. In 8 of 10 infants, alterations were visible at all ($n = 2$) or at selective ($n = 6$) electrode-positions.

Table 1 Clinical data of included patients

Case	AED before/during IV pyridoxine	Epilepsy syndrome	Seizure classification	Seizure type	Seizure onset	Age at IV pyridoxine
1	B, PB	—	C, G	Tonic clonic	0 d	12 d
2	B, LD, PB	—	S, G	Subtle	0 d	2 d
3	B, LD	—	S, G	Misc.	0 d	3 d
4	B, LTG, VPA	—	C, Pc	Subtle	0 d	8 m
5	B, PB	—	S, G	Tonic clonic	1 d	7 d
6	B, LD, PB	—	S, G	Tonic clonic	1 d	13 d
7	B, PB	—	C, PsG	Multif.myo.	3 m	3 m
8	B, LTG, VGB, VPA	West	C, G	Misc.	3 m	6 m
9	B, PB, VPA	—	C, PsG	Tonic clonic	4 m	7 m
10	B, VPA	West	C, G	Misc.	10 m	11 m

Abbreviations: AED = antiepileptic drug treatment; IV = intravenous; B = benzodiazepines; PB = phenobarbital; LD = lidocaine; LTG = lamotrigine; VPA = valproic acid; VGB = vigabatrin; C = cryptogenic; G = generalized; S = symptomatic; Pc = partial complex; PsG = partial secondarily generalized; multif.myo. = multifocal myoclonic; misc. = miscellaneous; d = postnatal day(s); m = postnatal months; — = absent.

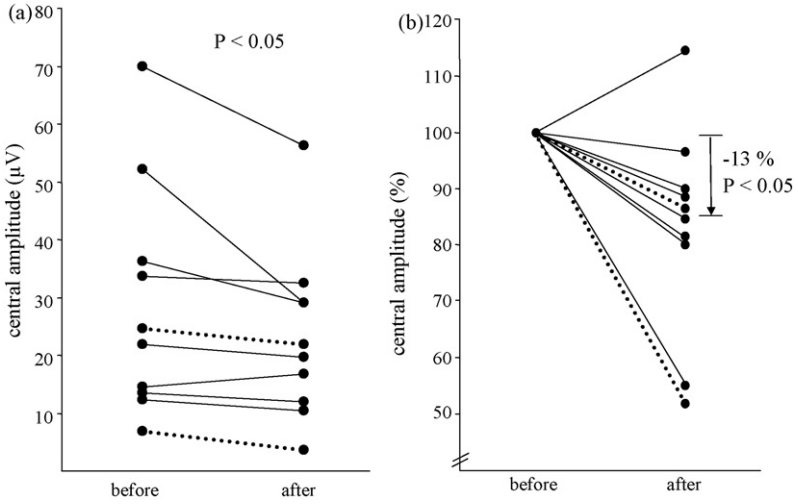

Figure 1 (a) Effect of pyridoxine IV on the EEG-amplitude at C3-C4. Before (horizontal axis, left) and after (horizontal axis, right) administration of pyridoxine-IV, central amplitude alterations (in μV, at vertical axis) are indicated. At central electrodes, amplitudes declined significantly by pyridoxine IV. (b) Relative effect of pyridoxine-IV on the EEG-amplitude at central electrodes. The pyridoxine-IV effect on amplitude is expressed as percentage of initial value, before pyridoxine administration. At central electrodes, pyridoxine-IV administration decreased the EEG-amplitude ($p < 0.05$). Median decline is indicated by an arrow. The two PR infants, cases 1 and 3, are separately indicated by dashed lines.

The pyridoxine-IV effect on total power was assessed for delta, theta and beta frequency-bands. In the delta frequency-band, total power declined ($p < 0.05$) at central and centro-temporal electrodes [C3-C4 (Fig. 2a): median −14% (−47 to +15%); C3-T3: median −14% (−47 to +6%)]. In the theta frequency-band, total power declined ($p < 0.05$) at central and frontal electrodes [C3-C4 (Fig. 2b): median −20% (−47 to +27%); F3-F4: median −14% (−58 to +25%)]. In the beta frequency-band, total power declined ($p < 0.05$) at central and centro-temporal electrodes [C3-C4 (Fig. 2c): median −11% (−54 to +21%); C4-T4: median −20% (−53 to +11%)].

EEG alterations were acquired while the child was asleep ($n = 7$) or awake ($n = 3$). EEG alterations by pyridoxine-IV during sleep consisted of declined

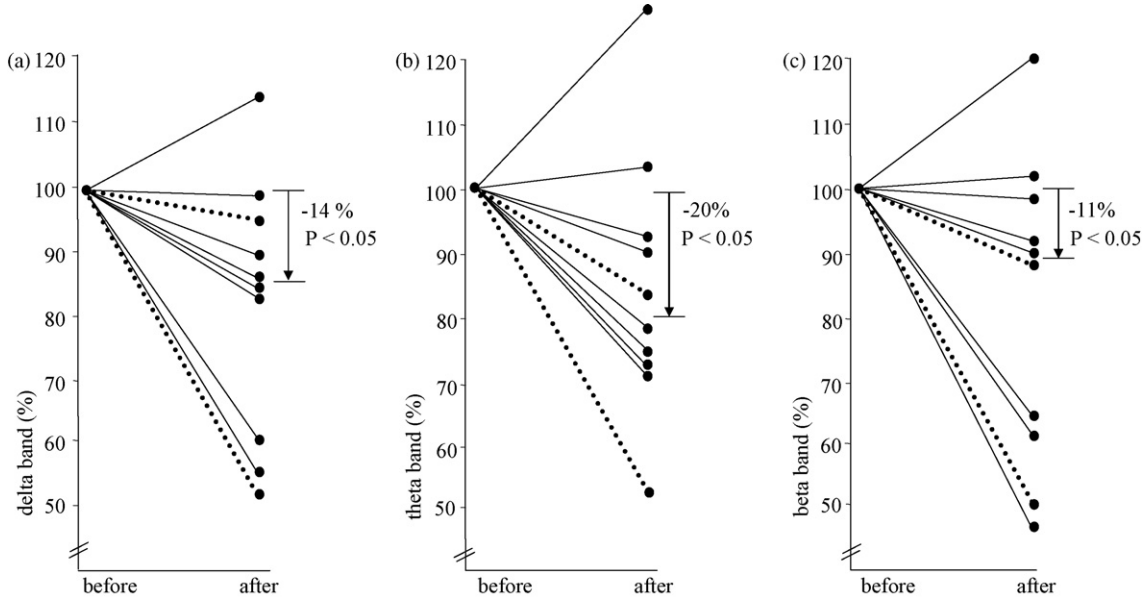

Figure 2 Relative effect of pyridoxine-IV on total power at C3-C4. The pyridoxine-IV effect on total power in delta (a), theta (b) and beta (c) frequency-bands is expressed as percentage of initial value, before pyridoxine administration. At central electrodes, pyridoxine-IV administration decreased the total power in the delta (a), theta (b) and beta (c) frequency bands ($p < 0.05$). Median declines are indicated by arrows. The two PR infants, cases 1 and 3, are separately indicated by dashed lines.

amplitudes [C3-C4, C3-T3 and F3-F4 ($p < 0.05$)] and total power [C3-C4 and C3-T3 in all frequency bands ($p < 0.02$); and F3-F4 in theta frequency-band]. After exclusion of PR, sleeping TRS-infants still showed similarly declined amplitude- and total power-parameters by pyridoxine-IV [C3-C4, C3-T3 and F3-F4 ($p < 0.05$)]. In the three infants that were awake, EEG alterations did not reach significance.

In five infants, the procedure of pyridoxine-IV administration provoked transient movements by the child (median duration 2 min). In these infants, EEG-fragments were only selected after transient movements had disappeared and the infant had returned to the original state. Comparison between infants with transient movement induction ($n = 5$) and without transient movement induction ($n = 5$) did not indicate differences regarding alterations in amplitude or total power by pyridoxine-IV.

In the total group, relative power was unaffected by pyridoxine-IV. The delta-, theta- and beta-frequency-bands before pyridoxine-IV were 41, 21 and 12% and after pyridoxine-IV 43, 21 and 12%, respectively. Stratification of the results according to vigilance levels [asleep ($n = 7$); awake ($n = 3$)] or according to transient movements [present ($n = 5$); absent ($n = 5$)], revealed an unaffected relative power by pyridoxine-IV.

Discussion

Among TRS-infants, PD/PR is recognized by the clinical response after empirical pyridoxine administration.[3] In TRS-infants with high seizure frequencies, pyridoxine-IV may cause prompt attenuation of seizures and normalization of the EEG, which is indicative for PD/PR.[2,3,15] However, (the time- and the extent-of) EEG normalization may vary among PD/PR-infants.[2,15] Furthermore, before EEG alterations can be ascribed to PD/PR, possible non-specific EEG responses (i.e. independent of PD/PR) should be excluded first. The present study in TRS-infants indicates that pyridoxine-IV causes a non-specific decline in EEG-amplitude and total power (which is independent of PD/PR). Since we carefully selected EEG fragments during identical vigilance states, declined EEG-amplitude and -total power could not be ascribed to arousal. This was also confirmed by the identical frequency spectra (relative power) before and after pyridoxine-IV, which indirectly reflects continuation of vigilance state. In young TRS-infants, these "non-specific" effects by pyridoxine-IV on EEG recordings have never been described before. In mice, pyridoxine-IV has been shown to affect EEG background activity after iatrogenic provocation of convulsions.[18] This effect was explained by relative attenuation of increased glutamate levels. However, our data in human TRS-infants do not allow speculations upon explanatory anti-glutamergic or GABA-ergic mechanisms. For instance, pyridoxine-IV did not enhance beta-activity (which is commonly observed after GABA-ergic activation) and CSF-GABA concentrations were normal.

In conclusion, pyridoxine-IV may affect EEG amplitude and total power in a non-specific way. In TRS-infants, these non-specific effects after pyridoxine-IV administration should be taken into account before declined amplitudes and total power can be attributed to PD/PR.

Acknowledgements

The authors wish to thank Mrs. Jane den Dunnen-Briggs for correction of English grammar; Mr. Lucas Dijck for his technical support; Mrs. Henrica Hooisma and Mrs. Marian Luursema for their administrative help.

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